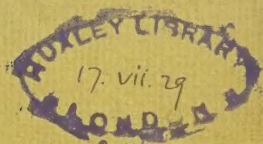


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INTERSPECIFIC HYBRIDIZATION IN *BRASSICA*. III. THE
CYTOLOGY OF F₁ HYBRID OF *B. CERNUA* AND
*B. NAPELLA*¹

Toshitaro MORINAGA

INTRODUCTION

It has already been reported in my previous papers (4, 5) that when *B. Napella*, characterized by nineteen chromosomes, is crossed with *B. chinensis* (or other allied species with ten chromosomes), ten bivalent and nine univalent chromosomes are found in the heterotypic division. I have also found that when *B. cernua* with eighteen chromosomes is crossed with *B. chinensis* or other allied species, ten bivalents and eight univalents are invariably observable in the heterotypic division (6). In both of these cases, as in the proto-type of *Drosera*-scheme hybrid, *D. longifolia* × *rotundifolia*, the most natural explanation of the mode of synaptic union in the heterotypic division seems to me to be that the whole ten chromosomes from one parental derivation conjugate with an equal number of those of the other parental derivation, leaving the surplus chromosomes as univalents (allosynapsis). If the above assumption is correct, both *B. Napella* and *B. cernua* should contain, in their reduced chromosome complements, ten chromosomes with strong affinity to those of *chinensis* or allied species. Thus when we cross

¹ Contributions from the Institute of Agronomy, Kyushu Imperial University, No. 14.

B. cernua and *B. Napella* we would expect the synaptic union between those *cernua*- and *Napella*-chromosomes, which have strong affinity to those of *chinensis*, thus producing ten bivalents. No prophecy, however, will be made as to whether or not any of the rest of the *cernua*-chromosomes find their synaptic mates among the remaining *Napella*-chromosomes.

From her extensive studies SINSKAIA (8) concludes that *B. pekinensis*, *B. chinensis* and *B. nipposinica* (*B. japonica*), all of Asiatic origin, are very closely allied to one another. *B. juncea* (*B. cernua*), though also of Asiatic origin, belongs to a different group from the former; while *B. napus*, originating in the Mediterranean region, is a member of a still another different group. *B. Napella* is a species rather recently introduced to Japan proper through Chôsen, and nothing is known about its origin. This species, however, externally resembles *B. napus* very closely, which fact suggests strongly its origin in that species.

Keeping these points in mind, I examined the karyological features in the F_1 hybrid *B. cernua* \times *B. Napella*. The results obtained will be briefly described here.

MATERIALS AND METHODS

Though the F_1 hybrids of *B. cernua* and *B. Napella* were first propagated successfully in my laboratory in 1925 (4), the cytological studies of the hybrids were left untouched until the spring of 1928, when I obtained newly seven F_1 hybrids of *B. cernua* \times *B. Napella*. All the seven F_1 plants grew vigorously, and showed an entirely uniform appearance, more closely resembling *Napella* than *cernua*. From these plants the root-tip was fixed in January-February with NAWASHIN's fixative, and anthers were fixed in March-April with either BOUIN's or BENDA's solution. The methods used were in general the same as described in my previous papers.

RESULTS OF EXPERIMENTS

On account of poor fixation no exact counting of somatic chromosomes was possible, though about thirty-seven chromosomes were observed in all root-tip cells examined by me.

In the heterotype metaphase of the pollen mother-cell, I invariably counted twenty-seven chromosomes, and I observed the formation of

ten gemini in the heterotype division. Actually ten bivalent chromosomes are easily discriminated from seventeen univalent ones, as the bivalents arrange themselves at the center of the equatorial plane, leaving the univalents scattered in the surface layer of the spindle substance. The univalents are occasionally found in the equatorial region, but are situated always outside the group of bivalent chromosomes (Figs. 1, 9, 10). The number of univalents in the equatorial region, or thereabout, differs greatly in different flowers. The position of univalents in the surface layer of the spindle is well illustrated in the polar view of the cell, especially when many of the univalents are situated at the same level (Text-fig. 1).

Text-fig. 1



Text-fig. 1. A cell in heterotype metaphase depicted at 3 different foci to show the position of univalents and bivalents. 1a, 5 univalents roughly in a ring (upper focus). 1b, 10 bivalents in the equator (middle focus), 1c, 9 univalents at the same focus in a ring and 3 univalents slightly upper or lower deviate somewhat from the ring (lower focus). $\times 4,000$.

Text-fig. 2



Text-fig. 2. A cell in heterotype anaphase depicted at 5 different foci to show the position of univalents and disjoined halves of bivalents. 2a, 4 univalents in a ring (upper-most focus). 2b, 10 halves of bivalents and two univalents (upper focus). 2c, 10 halves of bivalents

(lower focus). 2d, 9 univalents in a ring (lower-most focus), 2e, 2 univalents found in the next section. $\times 4,000$.

In the heterotype anaphase, the synaptic mates of all the bivalents disjoin from each other as in pure-bred species. Two stages, one following the other, are represented in Figs. 2-3. In both of these cells most of the univalents are found near the poles, while in the cell depicted in Fig. 4 most of them are situated near the equatorial region. This latter situation is met with in flowers in which a relatively high number of univalents are observed in or near the equatorial region in the metaphase. In either type of situation, the univalents keep their position, as in the metaphase, in the surface layer of the achromatic spindle (Fig. 4 and Text-fig. 2). The univalents far out of the equator move without splitting to the nearer pole, ultimately joining the halves of bivalents. Most univalents near or in the equator usually show wide split, though the real distribution of the halves to the opposite poles is accomplished in only relatively a few cases. Fig. 5 shows a cell in interkinesis; one daughter nucleus contains nineteen chromosomes, while the other contains twenty; one split univalent is found in the cytoplasm. The double nature of some chromosomes is noticed in the figure.

In the homotype metaphase, the chromosomes usually arrange themselves regularly in the equatorial plane (Fig. 6). From the results of eighty-four metaphasic countings the following table, showing the frequency distribution of chromosome number, was compiled:

Table I.

Frequency distribution of chromosome number in homotype metaphase.

Number of chromosomes		10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	Total
1. Chromosomes in equatorial plane only	}	0	0	0	0	3	1	4	14	19	15	14	6	7	1	0	0	0	0	84
2. All chromosomes on the spindle	}	0	0	0	0	2	1	3	11	15	20	13	9	7	3	0	0	0	0	84
3. Theoretical		0	0	0.1	0.4	1.5	4.0	7.9	12.5	15.6	15.6	12.5	7.9	4.0	1.5	0.4	0.1	0	0	—

In thirteen cells I counted the number of chromosomes in both homotypic plates, and the results are presented in Table II.

As shown in Fig. 7, the homotype anaphase is carried out very regularly. The average number of lagging chromosomes in the late anaphase was only 0.9. In an exceptional cases, however, I counted

as many as six lagging chromosomes. When there are comparatively many laggards in one homotype spindle, a relatively large number of them are found also in its sister spindle of the same cell. This irregularity seems to be connected with the behaviour of univalents in the preceding division. The total chromosome number in a mother-cell in homotype anaphase is about twice thirty-seven.

Table II.

Number of chromosomes in homotype metaphase.

Spindle I.		Spindle II.		Out of spindle	Total
On equatorial plate	Out of equatorial plate	On equatorial plate	Out of equatorial plate		
17	1	19	0	0	37
18	0	20	0	0	38
23	0	14	0	0	37
22	0	14	0	0	36 ?
17	0	20	0	0	37
14	1	21	2	1	39
19	0	18	1	0	38
18	0	19	0	0	37
16	3	20	1	0	40
17	0	19	1	0	37
17	1	18	0	0	36 ?
18	0	18	1	0	37
17	0	20	0	0	37
Average					
17.9	0.5	18.5	0.5	0.1	37.4

SOME IRREGULARITIES OBSERVED

1. In a certain locule containing mother-cells in heterotype metaphase I observed extraordinarily large metaphasic spindles though very

rarely. Fig. 8 presents such a cell in which more than ten bivalents and \pm thirty-four univalents are observable. A similar case observed in another *Brassica* may warrant the description here. In the horticultural variety of *B. Rapa*, "Keya-kabu," I found a group of a few pollen mother-cells markedly larger than the normal ones. Such large mother-cells possess supernumerary chromosomes, and in one such cell I could count about twenty chromosomes in the equatorial plane. The duplication of chromosome complement in this case seems to have occurred in late archesporial cell division to produce a group of tetraploid pollen mother-cells.

2. Three equatorial plates instead of two in the homotype metaphase were observed once in this study, showing an irregularity which is of rare occurrence.

3. Rarely dyads instead of tetrads were produced as the end results of the reduction division.

GENERAL REMARKS

From the foregoing description it is clear that the F_1 hybrids *B. cernua* \times *B. Napella* present $10_{II} + 17_I$ chromosomes in their heterotype metaphase. Thus I can say now on the basis of actual observations that the synaptic union occurs in ten pairs of chromosomes, and never in the remaining ones. I might further say that only those of *cernua* and *Napella* having a strong affinity to *chinensis* ones make synaptic union between them, when brought together by means of hybridization. The behaviour of bivalent and univalent chromosomes throughout the meiotic process is entirely similar to that observed in the interspecific *Brassica* hybrids reported in my previous papers (4, 5, 6). In short, the hybrid also belongs to the *Pilosella* type, and approximates the *Drosera* type. The same kind of chromosomal behaviour has also been observed in the hybrid of *Triticum vulgare* and *Aegilops cylindrica* (7, 2). The facts described in my previous papers and in the present one suggest the hypothesis that there are three dissimilar chromosomal sets, namely a, with ten chromosomes, b, with eight chromosomes, and c', with nine chromosomes, and that there is no affinity between chromosomes belonging to the different sets. All species with ten chromosomes contain only the set a, and *B. cernua* and *B. juncea*² contain

² The reduction division is carried normally in the F_1 hybrid of *B. cernua* and *B. juncea*.

the set a and b, while *B. Napella* contains the set a and c'. If *B. Napella* is derived from the Mediterranean species *B. napus*, the latter should contain the set a and c, where c is composed of eight chromosomes similar to those in c', but dissimilar to those in b which is found in Asiatic *B. cernua* with eighteen chromosomes. The chromosome complements in *B. oleracea* and in *Raphanus sativus* are both composed of nine chromosomes, but they probably belong to sets entirely different from each other (3, 1). There is no affinity between the *Raphanus*-set chromosomes and the chromosomes belong to the set a or b (1). The *oleracea*-set is now under investigation.

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EXPLANATION OF PLATES 6-7

All figures in plate 6 were drawn at a level lower than the table with the aid of Abbe's camera under a Zeiss apochromatic objective 1.5 mm. and compensation ocular 18, magnification 5,000. The original drawings have been reduced to two thirds the original size.

Fig. 1. Heterotype metaphase showing 10 bivalents and 17 univalents.

Figs. 2, 3. Heterotype anaphase, most univalents are located near poles.

Fig. 4. Heterotype anaphase, most univalents are located near equator.

Fig. 5. Interphase showing two daughter nuclei having 19 and 20 chromosomes respectively, and a split univalent out of nuclei.

Fig. 6. Homotype metaphase showing 18 and 20 chromosomes in each plate.

Fig. 7. Homotype anaphase showing its regular type of division.

Fig. 8. An abnormal cell in heterotype metaphase having supernumerary chromosomes.

All photographs in plate 7 were taken by "Phoku" using Zeiss apochromatic objective 1.5 mm. and negative lens L. All of them were enlarged 3.5 times in printing.

Figs. 9, 10. Heterotype metaphase. Fig. 9 is same cell as Fig. 1.

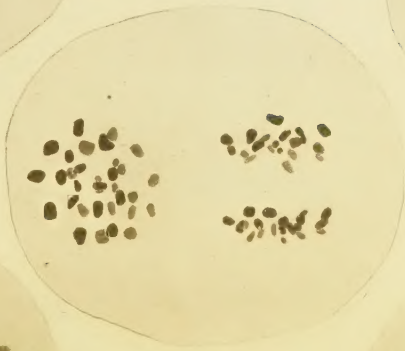
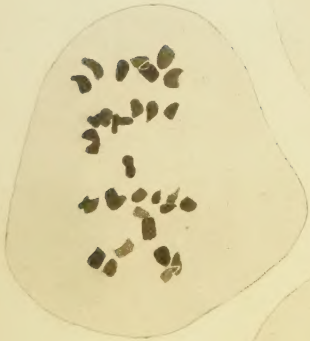
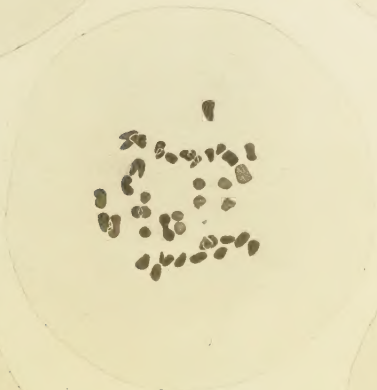
Figs. 11, 13. Heterotype anaphase, same cells as Fig. 2 and 3 respectively.

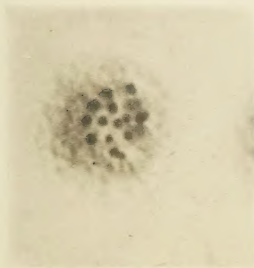
Figs. 12. Heterotype anaphase, same cell as Fig. 4; a at middle focus, b at upper focus and c at lower focus.

Fig. 14, 15. Homotype metaphase. Fig. 15 is same cell as Fig. 6.

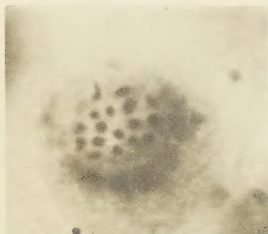
Fig. 16. Homotype anaphase, same cell as Fig. 7.

Fig. 17. Abnormal heterotype metaphase, same cell as Fig. 8.

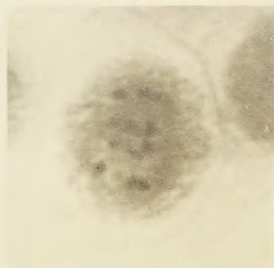




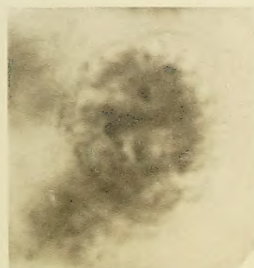
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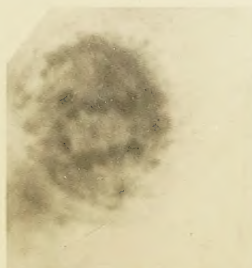
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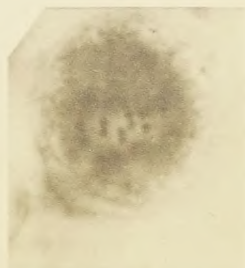
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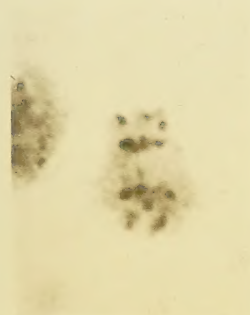
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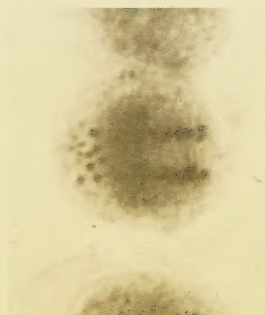
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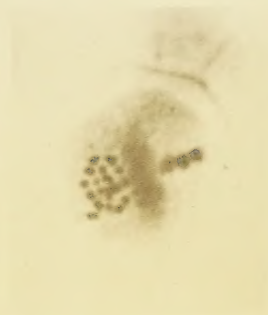
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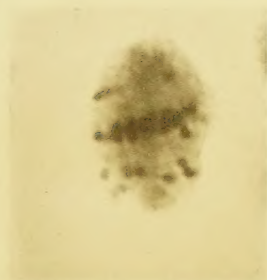
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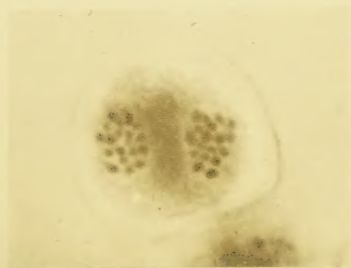
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